in the expression pattern of A341V in the absence or presence of KCNE1 were observed. Thus, the partial functional restoration by KCNE1 was not due to trafficking of A341V to the sarcolemma. To confirm that KCNE1 functionally rescued A341V, a mutant KCNE1, T58A that prevents the interaction of KCNE1 with KCNQ1, was cotransfected with A341V. T58A was unable to functionally restore A341V. Our studies showed an intriguing ability of KCNE1 to functionally rescue an LQTS-associated KCNQ1 mutant.

635-Pos

Differential Molecular Motions of KCNE Subunits in the I_{Ks} Channel Complex Detected by Substituted Cysteine-Accessibility Test and Disulfide-Trapping Experiments

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The I_{Ks} channel consists of a pore-forming KCNQ1 channel and auxiliary KCNE subunits: KCNE1 as the major determinant of I_{Ks} gating kinetics while KCNE2 as a regulator of I_{Ks} current amplitude. Knowledge about the structural basis of KCNQ1 modulation by these different KCNE subunits can help the design of KCNE-specific I_{Ks} modulators as antiarrhythmic agents. Recent studies showed that the extracellular-juxtamembranous region of KCNE1 could adopt highly flexible structures and make gating state-dependent contacts with the voltage-sensing domain (VSD) and pore-domain (PD) of KCNQ1. KCNQ1-KCNE1 interactions in this extracellular-juxtamembranous region are critical for the I_{Ks} channel function, as evidenced by congenital arrhythmia-associated mutations identified in this region. Whether KCNE2 can do the same is unclear. We systematically probe the functional role of the extracellular-juxtamembranous region of KCNE1 (positions 36-47) and KCNE2 (positions 39-50), using cysteine-scanning mutagenesis followed by cysteine-accessibility test and disulfide-trapping experiments. MTSET modification of KCNE1 40C-46C alters the voltage-dependence of I_{Ks} activation and, for 44C-46C, reduces the current amplitude. There is a gradient in MTSET modification rates, from fast-reacting (41C-42C) to slow-reacting (44C-46C), with 43C reporting state-dependent accessibility: fast-reacting in open-state & slow-reacting in closed-state. While extensive disulfide-bond partners are found between KCNE1 36C-43C and KCNQ1 144C-147C, no such disulfide-bond partners can be identified between the equivalent KCNE2 positions and KCNQ1 140C-148C. We propose that the KCNE transmembrane helices reside in the junction between VSDs and PD of the KCNQ1 channel, with a similar orientation (with respect to KCNQ1) and position (with respect to membrane bilayer). However, their extracellular-juxtamembranous regions can make differential contacts with KCNQ1, that contribute to their differential effects on the KCNQ1 channel function and provide an opportunity for the design of KCNE-specific I_{Ks} modulators.

636-Pos

Ether ȁ go-go Potassium Channels KCNH1 and KCNH5 Have Four Functional Orthologs in Danio Rerio

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The human ether à go-go channels KCNH1 and KCNH5 form a subgroup with unique functional properties within the eight-membered KCNH family of voltage-gated potassium channels. In mammals, the expression of both genes is mainly restricted to the brain, implying a role in electrical signaling of neurons. Heterologous expression in mammalian cells and *Xenopus* oocytes revealed that ether à go-go channels are sensitive to intracellular Ca^{2+}/calmodulin. The second functional characteristic of both channels is a pronounced slowing of activation kinetics upon binding of extracellular divalent cations. To elucidate the physiological relevance of such properties, an adequate model organism would be desirable. Here we studied the expression of ether à go-go orthologs in zebrafish. Due to a whole-genome duplication during evolution of teleost fish, mammalian genes can have two functional orthologs in zebrafish. However, for the majority of duplicated genes, one gene copy is lost or present as nonfunctional pseudogene only. Using *in silico* screening of genome databases and cloning from reverse transcribed mRNA, we could show that for each of the two human ether à go-go channels two functional orthologs exist in zebrafish. Upon expression in *Xenopus* oocytes, all four genes generated functional channels with current-voltage relationships similar to the human orthologs, characterized by a very negative threshold of the activation voltages. A more pronounced slowing of activation kinetics in the presence of extracellular Mg^{2+} ions clearly distinguished KCNH5 and its two fish orthologs from human KCNH1 and the corresponding fish channels. In summary, the structural and functional conservation between human and fish *ether à go-go* channels makes zebrafish a promising model, but gene duplication must be taken into account. This may also be relevant for the closely related paralogs ERG and ELK.

637-Pos

S4 Arginines Make Unique Contributions to Voltage Dependent Gating Due to Electrostatic Interactions and the Membrane Potential

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Conserved positively charged arginines in the fourth transmembrane segment (S4) of Kv channels are responsible for imparting voltage sensitivity to the channel. There are several forces that may influence these arginines including the membrane potential and electrostatic interactions with countercharges. In Shaker channels, the first four arginines are the primary gating charges that sense the membrane potential. Kv7.1 has fewer positively charged S4 residues than Shaker, notably with the third arginine in Shaker replaced by a glutamine (Q3). Further loss of charge induced by charge reversal at R1 (R1E) in Kv7.1 results in constitutively activated channels, perhaps due to insufficient charge in S4. Consistent with this idea, introduction of a positive charge at Q3 (Q3R) can restore voltage dependent activation to R1E, suggesting that Q3R may substitute for the loss of gating charge at R1E. In a related study, we have demonstrated in Kv7.1 channels that residues corresponding to the first four arginines in Shaker channels (R1-R4) interact sequentially with the first conserved glutamate in S2 (E1) during gating. Here we show via intragenic suppression that S4 arginines also interact electrostatically with the second conserved glutamate in S2 (E2), and these electrostatic interactions play an important role in voltage sensing of S4. Therefore, a network of electrostatic interactions and the membrane potential act on S4 arginines, and the balance of these forces stabilize the conformation of the voltage sensor at different states. The combination of these interactions acts uniquely on each arginine such that each arginine plays a different role in voltage dependent gating. In Kv7.1, the first two arginines (R1, R2) stabilize the resting state while the last three charged residues (R4, H5, R6) stabilize the activated state.

638-Pos

Non-Toxin Gating Modifiers Reveal the Promiscuous Nature of the Voltage Sensor of Kv7.2 and TRPV1 Channels

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Voltage-sensitive cation channel architecture consists of two main structural modules, the voltage sensor domain (VSD) and the pore domain. The VSDs are the target of various gating-modifier toxins and their paddle motifs are modular and transferable structures. Here we show that NH17 and NH29, two new Kv7.2 channel blocker and opener, respectively, act as non-toxin gating modifiers. Mutagenesis and modeling data suggest that in Kv7.2, NH29 docks to the external groove formed by the interface of helices S1, S2 and S4 in a way, which stabilizes the interaction between two conserved charged residues in S2, and S4, known to interact electrostatically, in the open state of Kv channels. Reflecting the promiscuous nature of the VSD, NH29 is also a potent blocker of TRPV1 channels, a feature similar to that described for tarantula toxins. TRPV1 channels appear to be weakly voltage-dependent. However, NH29 changes the linear TRPV1 current-voltage relation obtained with capsaicin, to an outwardly rectifying shape. Interestingly, mutations in linker S3-S4 of the TRPV1 VSD are significantly more resistant to the inhibitory effect of NH29. While compound NH17 potently blocks Kv7.2 channels, it sensitizes the TRPV1 current activated by capsaicin. Mutations in linker S3-S4 switch the TRPV1 sensitizing action of NH17 to a potent inhibition. Subtle modifications in the VSD or in the chemical structure of the molecule drastically change the attributes of the gating-modifier, thereby stabilizing the channel in either the closed or the open state. Data indicate that the novel compounds may operate via a voltage-sensor trapping mechanism similar to that suggested for scorpion and sea anemone toxins. Thus, the VSDs of Kv and TRPV1 channels are promiscuous and share some common structural and biophysical features.

639-Pos

Molecular Mechanism of Slow Kv7.1 Inactivation

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The molecular mechanisms involved in slow C-type inactivation are not clear yet but may involve structural rearrangements in the outer pore domain

paralleled by impaired K^+ coordination in the selectivity filter (Choi et al., 1991; Hoshi et al., 1990; Lopez-Barneo et al., 1993). The mechanism of KCNQ1 inactivation and its modulation by external K^+ are dissimilar to the mechanism described for C-type inactivation in *Shaker*-like K^+ channels (Gibor et al., 2007). Further, inactivation of wild-type (WT) KCNQ1 channels becomes evident only in the characteristic hooked tail currents which reflect recovery from inactivation (Abitbol et al., 1999; Pusch et al., 1998; Tristani-Firouzi and Sanguinetti, 1998). We use a combination of functional-structural analysis combined with mathematical and 3D-structural modeling to gain insights into the structural rearrangements during KCNQ1-inactivation. We show that the Kv7.1 α-subunits act in a concerted way to initiate KCNQ1-inactivation.

640-Pos

Non-Obligatory Gating of Kv7.1 Potassium Channel

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The response of voltage-dependent K+ (Kv) channels to a change in membrane voltage involves a molecular device, which couples voltage-driven conformational changes to gate opening and closing within the channel's conduction pathway. To further our understanding of the coupling choreography we have studied how changes in coupling strength instigate the Kv channel to open before and after voltage-sensor activation. We used single and double mutations in a Kv channel pore domain to analyze coupling sensitive sites. We observed in the mutational effects a correlation between coupling strength and non-obligatory Kv channel gating that is well described with a four-state allosteric gating model. Mapping the data onto known Kv channel structures showed that coupling-sensitive amino acid residues are strategically clustered to a small area between pore gate and the interface of pore and voltage sensors. We propose that the physical contact at the interface between voltage sensor and pore domain is an important determinant of altered coupling strengths leading to obligatory and non-obligatory Kv channel gating.

641-Pos

Diclofenac Activates Kv7.4 and Inhibits Kv7.5 Potassium Channels Heterologously Expressed in A7r5 Vascular Smooth Muscle Cells

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Members of the KCNQ (Kv7) voltage-gated potassium channel gene family are differentially expressed through the body. Five Kv7 subtypes play major roles in regulation of membrane potential and cell excitability within different tissues. Well known functions of Kv7.1 in cardiac action potential duration, Kv7.2/7.3 in neuronal excitability, Kv7.4 in hearing and an emerging role of Kv7.5 in vascular tone, increases the demand for channel modulating drugs that exhibit selectivity among Kv7 subtypes. Diclofenac, an anti-inflammatory drug, was found to be a novel Kv7.2/7.3 channel opener and was used as a template to synthesize new activators and inhibitors of neuronal KCNQ channels (Kv7.2/7.3 and Kv7.4). We compare affects of diclofenac on human Kv7.4 and Kv7.5 currents using A7r5 rat aortic smooth muscle cells as an expression system and perforated patch-clamp techniques. Diclofenac, at 100∈ 1/4M, increased maximal conductance of Kv7.4 channels by 1.5-fold and induced a negative shift in the activation curve (by 9mV). Surprisingly, the same concentration of diclofenac (100∈ 1/4M) reduced maximal conductance of Kv7.5 channels by 2-fold, but also induced a robust negative shift in the activation curve (by 30mV). For the both Kv7.4 and Kv7.5, application of diclofenac (100∈ 1/4M) reduced the deactivation rate of the current. For Kv7.4, the deactivation rate was 1.7-fold slower in the presence of diclofenac, independent of the voltage in the range from −120mV to −90mV. In contrast, for Kv7.5 the reduced deactivation rate in the presence of diclofenac was voltage-dependent, changing linearly from 2-fold at −120mV to 4-fold at −90mV. These differences in diclofenac action on two members of the Kv7 channel family may reflect structural differences between Kv7.4 and Kv7.5 and make diclofenac a useful tool to distinguish between Kv7.4 and Kv7.5 currents in native tissues.

Ca-Activated Channels

642-Pos

Cholesterol Depletion Alters Amplitude and Pharmacology of Vascular Calcium-Activated Chloride Channels

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Calcium-activated chloride channels (CACCs) share common pharmacological properties with SLO (KCNMA1)-encoded large conductance K^+ channels ($K_{Ca}1.1$) and it has been suggested they may co-exist in a macromolecular complex (Greenwood, I. A., and Leblanc, N. *Trends Pharmacol Sci*28: 1-5, 2007; Saleh *et al.* J Pharmacol Exp Ther 321: 1075-1084, 2007; Sones *et al. Br J Pharmacol* 158: 521-531, 2009). As $K_{Ca}1.1$ channels are known to localise to cholesterol and caveolin-rich lipid rafts (caveolae) the present study investigated whether Ca^{2+}-sensitive Cl^- currents in vascular myocytes were affected by the cholesterol depleting agent Beta-methyl cyclodextrin (Beta-MCD). Calcium-activated chloride and potassium currents were recorded from single murine portal vein myocytes in whole cell voltage clamp. Western blot was undertaken following sucrose gradient ultracentrifugation using protein lysates from whole portal veins. Ca^{2+}-activated Cl^- currents were augmented by Beta-MCD with a rapid time-course ($t_{0.5}$ = 1.8 min). Beta-MCD had no effect on the bi-modal response to niflumic acid or anthracene-9-carboxylate but completely removed the inhibitory effects of the $K_{Ca}1.1$ blocker paxilline and the stimulatory effect of the $K_{Ca}1.1$ activator NS1619. Discontinuous sucrose density gradients followed by Western blot analysis revealed that $K_{Ca}1.1$ was present in lipid fractions, co-localising with lipid raft markers caveolin and flotillin-2. The newly identified candidate for calcium-activated chloride channels TMEM16A, co-localised to the same fractions as $K_{Ca}1.1$. These data reveal that CACC properties are influenced by lipid raft integrity. The results also provide a structural basis explaining the intimate functional interaction that exist between $K_{Ca}1.1$ and CACCs in generating STOCs and STICs and how they regulate resting membrane potential and tone in vascular myocytes.

643-Pos

Ca2$^+$-Activated Cl- Currents of Pulmonary Artery Smooth Muscle Cells are Enhanced in Monocrotaline-Induced Pulmonary Arterial Hypertension in the Rat

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Pulmonary arterial hypertension (PAH) in humans is defined by a pulmonary artery pressure (PAP) exceeding 25 mm Hg at rest, and 30 mm Hg during physical activity. Three major factors contribute to elevating PAP in PAH patients: 1) enhanced vasoconstriction; 2) reduction of lumen diameter due to remodeling of the arterial wall; and 3) enhanced clot formation. It has been recently suggested that Cl^- currents can regulate proliferation of cultured rat pulmonary artery (PA) smooth muscle cells (Liang et al., *Hypertension* 54: 286-293, 2009). The purpose of the present study was to determine if Ca^{2+}-activated Cl^- currents ($I_{Cl(Ca)}$) are altered in PA smooth muscle cells from monocrotaline(MCT)-treated rats. Aged-matched male rats were either injected with saline or a single dose of MCT (50 mg/kg) to induce PAH, and the animals from both groups were sacrificed after 3 weeks. Rats treated with MCT displayed an increase in right ventricular weight with no change in left ventricular and septum weights, consistent with PAH. Patch clamp experiments revealed that the cell capacitance, an index of cell surface, of PA cells from MCT-injected rats was 40% greater than that of cells from saline-injected rats. Time- and voltage-dependent $I_{Cl(Ca)}$ elicited by 500 nM internal free Ca^{2+} (buffered with 10 mM BAPTA) displayed outward rectification in both groups but was more than ~ 2-fold larger in the MCT *vs.* saline group. In both groups, the current ran down over time but significantly more on a percentage basis in MCT than control cells. In conclusion, the properties and regulation of $I_{Cl(Ca)}$ appear to be altered in a validated animal model of PAH and these results suggest that this anion current may represent a new therapeutic target.

644-Pos

The Interaction of Antrhacene-9-Carboxylic Acid with Calcium-Activated Chloride Channels is Influenced by the State of Global Phosphorylation in Pulmonarty Artery Smooth Muscle Cells

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Ca^{2+}-dependent Cl^- currents (I_{ClCa}) are inhibited by phosphorylation in arterial smooth muscle cells. We recently reported that niflumic acid (NFA), an inhibitor of I_{ClCa}, is less efficacious at blocking these currents in conditions promoting phosphorylation (Wiwchar *et al.*, *Br J Pharmacol*, in press, 2009). This